

APPENDIX I

1. (once amended) An isolated nucleic acid encoding a polypeptide monomer of a pH sensitive potassium channel, the monomer:

(i) having a unit conductance of approximately 80-120 pS when the monomer is in a functional tetrameric form of a potassium channel and is expressed in a *Xenopus* oocyte;

(ii) having increased activity above approximately intracellular pH of 7.1;
and

(iii) specifically binding to polyclonal antibodies generated against a polypeptide comprising an amino acid sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:16, or SEQ ID NO:18.

2. (as filed) An isolated nucleic acid of claim 1, wherein the nucleic acid encodes mSlo3.

3. (as filed) An isolated nucleic acid of claim 1, wherein the nucleic acid encodes hSlo3.

4. (as filed) An isolated nucleic acid of claim 1, wherein the nucleic acid encodes SEQ ID NO:1.

5. (as filed) An isolated nucleic acid of claim 1, wherein the nucleic acid encodes SEQ ID NO:16 or 18.

6. (once amended) An isolated nucleic acid of claim 1, wherein the nucleic acid selectively hybridizes under moderate stringency hybridization conditions, which end with a

wash step at 45°C in a solution comprising 1x SSC, to a nucleic acid comprising a nucleotide sequence of SEQ ID NO:2.

7. (once amended) An isolated nucleic acid of claim 1, wherein the nucleic acid selectively hybridizes under moderate stringency hybridization conditions, which end with a wash step at 45°C in a solution comprising 1x SSC, to a nucleic acid comprising a nucleotide sequence of SEQ ID NO:4, SEQ ID NO:17, or SEQ ID NO:19.

8. (as filed) An isolated nucleic acid sequence of claim 1, wherein the nucleic acid has a nucleotide sequence of SEQ ID NO:2.

9. (as filed) An isolated nucleic acid sequence of claim 1, wherein the nucleic acid has a nucleotide sequence of SEQ ID NO:4, SEQ ID NO:17, or SEQ ID NO:19.

10. (as filed) An isolated nucleic acid of claim 1, wherein the nucleic acid is amplified by primers that selectively hybridize under stringent hybridization conditions to the same sequence as the primer sets selected from the group consisting of:

CTCGAACTCCCTAAAATCTTACAGAT (SEQ ID NO:8) and
TTCCGTTGAGCCAGGGGTCACCAGAATT (SEQ ID NO:9);
TCTGCTTTGTGAAGCTAAATCT (SEQ ID NO:10) and
TTTCAAAGCCTCTTTAGCGGTAA (SEQ ID NO:11); and
TTATGCCTGGATCTGCACTCTACATG (SEQ ID NO:12) and
ATAGTTTCCGTCTACTACCGAAA (SEQ ID NO:13).

11. (as filed) An isolated nucleic acid of claim 1, wherein the nucleic acid is amplified by primers that selectively hybridize under stringent hybridization conditions to the same sequence as the primer sets selected from the group consisting of:

GGCAGCGCTCATTCTTTCCTCCTT (SEQ ID NO:14) and

TGCCCAAACCTCAACCCAAAATA (SEQ ID NO:15).

12. (once amended) An isolated nucleic acid encoding at least 25 contiguous amino acids from a polypeptide having an amino acid sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:16, SEQ ID NO:18; wherein the nucleic acid encodes a pH sensitive potassium channel polypeptide monomer.

13. (once amended) The isolated nucleic acid of claim 12, wherein said nucleic acid encodes a pH sensitive potassium channel polypeptide monomer having:

(i) a unit conductance of 80-120 pS when the monomer is in a functional tetrameric form of a potassium channel and is expressed in a *Xenopus* oocyte; and

(ii) increased activity above an intracellular pH of 7.1;

wherein said nucleic acid

selectively hybridizes under moderate stringency hybridization conditions, which end with a wash step at 45°C in a solution comprising 1x SSC, to a nucleic acid comprising a nucleotide sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:17, SEQ ID NO:19.

14. (once amended) An isolated nucleic acid encoding a polypeptide monomer of a pH sensitive potassium channel, the monomer:

(i) having a core domain that has greater than 60% amino acid sequence identity to a polypeptide comprising amino acids 35-641 of SEQ ID NO:1 as measured using a sequence comparison algorithm;

(ii) having a unit conductance of approximately 80-120 pS when the monomer is in a functional tetrameric form of a potassium channel and is expressed in a *Xenopus* oocyte; and

(iii) having increased activity above approximately intracellular pH of 7.1.

15. (as filed) An isolated nucleic acid of claim 14, wherein the Slo3 has a sequence of SEQ ID NO:1.

16. (as filed) An isolated nucleic acid of claim 14, wherein the Slo3 has a sequence of SEQ ID NO:16 or SEQ ID NO:18.

17. (withdrawn) An isolated polypeptide monomer of a pH sensitive potassium channel, the monomer:

(i) having a calculated molecular weight of between 120-156 kDa;

(ii) having a unit conductance of approximately 80-120 pS when the monomer is in a functional tetrameric form of a potassium channel and is expressed in a *Xenopus* oocyte;

(iii) having increased activity above approximately intracellular pH of 7.1;

and

(iv) specifically binding to polyclonal antibodies generated against SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:16 OR SEQ ID NO:18.

18. (withdrawn) An isolated monomer of claim 17, wherein the monomer has an amino acid sequence of mSlo3.

19. (withdrawn) An isolated monomer of claim 17, wherein the monomer has an amino acid sequence of hSlo3.

20. (withdrawn) An isolated monomer of claim 17, wherein the monomer has an amino acid sequence of SEQ ID NO:1.

21. (withdrawn) An isolated monomer of claim 17, wherein the monomer has an amino acid sequence of SEQ ID NO:16 or SEQ ID NO:18.

22. (withdrawn) An antibody that selectively binds to mSlo3.
23. (withdrawn) An antibody of claim 22, wherein the mSlo3 has an amino acid sequence of SEQ ID NO:1.
24. (withdrawn) An antibody that selectively binds to hSlo3.
25. (withdrawn) An antibody of claim 24, wherein the hSlo3 has an amino acid sequence of SEQ ID NO:16 or SEQ ID NO:18.
26. (once amended) An expression vector comprising a nucleic acid encoding a polypeptide monomer of a pH sensitive potassium channel, the monomer:
 - (i) having a unit conductance of approximately 80-120 pS when the monomer is in a functional tetrameric form of a potassium channel and is expressed in a *Xenopus* oocyte;
 - (ii) having increased activity above approximately intracellular pH of 7.1;and
 - (iii) specifically binding to polyclonal antibodies generated against a polypeptide comprising an amino acid sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:16, or SEQ ID NO:18.
27. (as filed) A host cell transfected with the vector of claim 26.
28. (withdrawn) A method for identifying a compound that increases or decreases ion flux through a pH sensitive potassium channel, the method comprising the steps of:
 - (i) contacting the compound with a eukaryotic host cell or cell membrane in which has been expressed a pH sensitive potassium channel monomer polypeptide:

(a) having a calculated molecular weight of between 120-156 kDa;
(b) having a unit conductance of approximately 80-120 pS when the monomer is in the functional tetrameric form of a potassium channel and is expressed in a *Xenopus* oocyte; and

(c) specifically binding to polyclonal antibodies generated against SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:16 or SEQ ID NO:18; and

(ii) determining the functional effect of the compound upon the cell or cell membrane expressing the pH sensitive potassium channel.

29. (withdrawn) A method of claim 28, wherein the increased or decreased flux of ions is determined by measuring whole cell conductance.

30. (withdrawn) A method of claim 28, wherein the pH sensitive potassium channel monomer polypeptide is recombinant.

31. (withdrawn) A method of claim 28, wherein the pH sensitive potassium channel monomer polypeptide is mSlo3.

32. (withdrawn) A method of claim 28, wherein the pH sensitive potassium channel monomer polypeptide is hSlo3.

33. (withdrawn) A method of claim 28, wherein the pH sensitive potassium channel monomer polypeptide has an amino acid sequence of SEQ ID NO:1.

34. (withdrawn) A method of claim 28, wherein the pH sensitive potassium channel monomer polypeptide has an amino acid sequence of SEQ ID NO:16 or SEQ ID NO:18.

35. (withdrawn) A method of detecting the presence of Slo3 in mammalian tissue, the method comprising the steps of:

- (i) isolating a biological sample;
- (ii) contacting the biological sample with a Slo3-specific reagent that selectively binds to Slo3; and,
- (iii) detecting the level of Slo3-specific reagent that selectively associates with the sample.

36. (withdrawn) A method of claim 35, wherein the Slo3 specific reagent is selected from the group consisting of: Slo3 specific antibodies, Slo3 specific oligonucleotide primers, and Slo3 nucleic acid probes.

37. (withdrawn) A method of claim 35, wherein the sample is from a human.

38. (withdrawn) In a computer system, a method of screening for mutations of Slo3 genes, the method comprising the steps of:

- (i) receiving input of at least about 30 nucleotides of first nucleic acid sequence encoding a pH sensitive potassium channel protein having a nucleotide sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:17, or SEQ ID NO:19 and conservatively modified versions thereof;
- (ii) comparing the first nucleic acid sequence with a second nucleic acid sequence having substantial identity to the first nucleic acid sequence; and
- (iii) identifying nucleotide differences between the first and second nucleic acid sequences.

39. (withdrawn) The method of claim 38, wherein the second nucleic acid sequence is associated with a disease state.

40. (withdrawn) In a computer system, a method for identifying a three-dimensional structure of Slo3 proteins, the method comprising the steps of:

(i) receiving input of at least about 10 amino acids of an amino acid sequence of a pH sensitive potassium channel monomer or at least about 30 nucleotides of a nucleotide sequence of a gene encoding the protein, the protein having an amino acid sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:16, SEQ ID NO:18, and conservatively modified versions thereof; and

(ii) generating a three-dimensional structure of the protein encoded by the amino acid sequence.

41. (withdrawn) The method of claim 40, wherein said amino acid sequence is a primary structure and wherein said generating step includes the steps of:

(i) forming a secondary structure from said primary structure using energy terms encoded by the primary structure; and

(ii) forming a tertiary structure from said secondary structure using energy terms encoded by said secondary structure.

42. (withdrawn) The method of claim 40, wherein said generating step includes the step of forming a quaternary structure from said tertiary structure using anisotropy terms encoded by the tertiary structure.

43. (withdrawn) The method of claim 41, wherein said generating step further includes the step of forming a quaternary structure from said tertiary structure using anisotropy terms encoded by the tertiary structure.

44. (withdrawn) The method of claim 40, further comprising the step of identifying regions of the three-dimensional structure of the protein that bind to ligands and using the regions to identify ligands that bind to the protein.

45. (new) The nucleic acid of claim 1, wherein the nucleic acid encodes a polypeptide monomer having a calculated molecular weight of between 120-156 kDa.

46. (new) The nucleic acid of claim 1, wherein the nucleic acid encodes a polypeptide monomer forming a homomeric potassium channel.

47. (new) The nucleic acid of claim 1, wherein the nucleic acid encodes a polypeptide monomer forming a heteromeric potassium channel.

48. (new) An isolated nucleic acid encoding a polypeptide monomer of a pH sensitive potassium channel, the monomer:

(i) having a unit conductance of approximately 80-120 pS when the monomer is in a functional tetrameric form of a potassium channel and is expressed in a *Xenopus* oocyte; and

(ii) having increased activity above approximately intracellular pH of 7.1; wherein said nucleic acid selectively hybridizes under highly stringent hybridization conditions, which end with a wash step at 65°C in a solution comprising 0.2x SSC and 0.1% SDS, to a nucleic acid comprising a nucleotide sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:17, SEQ ID NO:19.